Docket No.: D0590.70011US02
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Geert Karel Maria Plaetinck et al.
Serial No.:	10/826,522
Confirmation No.:	2890
Filed:	April 16, 2004
For:	CHARACTERISATION OF GENE FUNCTION USING DOUBLE STRANDED RNA INHIBITION
Examiner:	D. H. Shin
Art Unit:	1635

Declaration of Dr. Erwin Sablon Under 37 C.F.R. § 1.132

1. I have held the position of Director Diagnostics Development & Alliance Management at Biocartis in Lausanne, Switzerland since June 2010. Prior to that, I was Director, Project Management at Ablynx NV in Gent, Belgium from October 2008 to June 2010. Prior to that, I was Associate Director, Project Management at Ablynx NV. Prior to that, I was Head, Infectious Diseases Dept., R&D at Innogenetics in Gent, Belgium from 2005-2008; Head, Virology Dept, R&D at Innogenetics NV from 1998 - 2005; and Head, Microbiology & bioprocessing unit at Innogenetics NV from 1996 - 1998, Group Leader microbial expression technology and protein engineering at Innogenetics NV from 1992-1996, and scientist at the Microbiology Dept., Innogenetics NV from 1990-1992. I received a Ph.D. degree in Molecular Biology from the University of Gent (Belgium) in 1990. I have published extensively and am knowledgeable in the area of molecular DNA and RNA engineering and microbial expression technologies. My curriculum vitae is attached as Exhibit 1

2. I have reviewed the Office Action dated June 29, 2010 and it is my understanding that the claims are rejected as being obvious in view of the following combinations of references:

- Timmons et al. (*East Coast Worm Meeting*, abstract 180) in view of McAllister et al. (US Patent 5,017,488), Conkling et al. (US Patent 5,459,252), and Talkad et al. (J. Bacteriol. 135:528-541, 1978); and
- Fire et al. (WO99/32619), in view of Graham (US Patent 6,573,099), Ely et al. (US Patent 5,837,848) and Talkad et al. (J. Bacteriol. 135:528-541, 1978).

3. I am not an inventor of the above-identified patent application. I am not an author of any of the articles referenced in the Office Action in rejecting the claims, and am not an inventor of any of the patents referenced in the Office Action in rejecting the claims.

4. I have been informed that the standard used for determining whether claims are unpatentable for obviousness is that: (1) the combination of prior art references must teach all of the elements of the claimed invention; (2) a reason to combine teachings of the references must exist; and (3) there was reasonable expectation of success in obtaining the claimed invention by combining the elements from the prior art.

5. The person of ordinary skill in the art is a graduate or post-graduate scientist with several years of research experience in molecular biology and microbial expression technologies, preferably in *in vitro* and *in vivo* expression techniques.

6. According to the claims of the above-identified application, the invention relates to methods for down-regulating the expression of a gene of interest in *C. elegans* by feeding *C. elegans* with a micro-organism that expresses dsRNA corresponding to the gene of interest. The microorganism comprises an expression vector that comprises a DNA sequence corresponding to the gene of interest, in which the expression vector comprises a promoter or promoters flanking said DNA sequence such that the promoter or promoters initiate transcription of said DNA sequence to produce double stranded RNA upon binding of a transcription factor to said promoter or promoters. Upon production of the dsRNA by the microorganism and feeding the microorganism to *C. elegans*, the expression of the gene of interest in *C. elegans* is downregulated.

7. The McAllister et al. patent describes vectors contain a multiple cloning site flanked by two phage RNA polymerase promoters positioned to express either strand of a DNA molecule inserted in the multiple cloning site. These vectors have SP6 and T3 bacteriophage promoters or

T7 and T3 bacteriophage promoters. See the McAllister et al patent throughout, such as at col. 1, line 63 to col. 2, line 4.

8. The vectors described in the McAllister et al. patent are used for *in vitro* transcription of RNA of one strand of a DNA sequence, such as for making RNA probes. See claim 7 of the McAllister et al patent, which states that "RNA strands complementary to either one of the strands of the inserted DNA sequence" are produced by transcription These flanking promoters are not the same.

9. The rationale of using the vectors described in the McAllister et al. patent is that one can generate *in vitro*, using the appropriate phage RNA polymerase, either sense or antisense transcripts from the same vector.

10. Because the purpose of the vectors described in the McAllister et al. patent is to generate synthetic RNA probes (see Background section, col. 1), it is crucial that only one strand becomes transcribed (either sense or antisense, but not both). This is brought about by one of two methods. First, one can linearize the vector with a particular restriction endonuclease that cuts between the insert and one of the flanking promoters in order to ensure transcription of only one strand. This is typically the case when both flanking promoters are of the same type. Second, for vectors in which the two flanking promoters are of different origin, one can use only one promoter-specific phage RNA polymerase in the *in vitro* transcription reaction.

11. The vectors described in the McAllister et al. patent were clearly not intended to simultaneously produce transcripts from both directions.

12 On page 5 of the Office Action, the Examiner stated that: "the vector system of McAllister et al. can produce the sense orientation DNA sequence and at the same [*sic*, same time] can produce the antisense ... orientation DNA sequence, thereby being capable of simultaneously transcribing both RNA 'strands' of the inserted DNA sequence." For the purposes of commenting on the Examiner's statement, I have assumed that the Examiner meant

to indicate that the McAllister vectors are capable of producing sense and antisense orientation RNA sequences by transcription of both DNA "strands" of a DNA sequence inserted in the McAllister vectors.

13. I respectfully disagree with the statements of the Examiner made in the Office Action as referenced in paragraph 12. The Examiner's statement that the McAllister vectors are capable of producing sense and antisense orientation RNA sequences by transcription of both strands of an inserted DNA sequence cannot be concluded from the McAllister et al. patent.

14. There is nothing in the McAllister et al. patent to suggest to the skilled person that double stranded RNA could be made. Indeed, a mentioned use is for synthesizing RNA probes of high sensitivity. See col. 3, lines 44-46. RNA probes are known to the skilled person to be single-stranded.

15. In contrast to the vectors described in the McAllister et al. patent, the above-identified application describes the use of bidirectional expression vectors for *in vivo* generation in *E. coli* of double stranded RNA. Such a use would not have been suggested to the person of ordinary skill in the art by the McAllister et al. patent for the reasons stated above. Moreover, such type of vectors, having opposite RNA polymerase promoters, have been well known to and universally used by molecular biologists since the mid 1980s, when I was performing my PhD. The use of this type of vectors for "*in vitro*" production of both strands (ds) RNA in a microorganism was not even anticipated by any molecular biologist at that time, not until the discovery thereof by the inventors of the present invention in the late 1990s.

16. On page 8, first paragraph, of the Office Action, the Examiner stated that Fire et al provides adequate support for "a target inhibition method in *C. elegans* with an expression vector that synthesizes two separate complementary RNA strands that form an RNA duplex, wherein the synthesis of the two RNA strands is driven by bacteriophage polymerase promoters such as T3, T7 and SP6 promoters."

17. On page 9 of the Office Action, the Examiner stated that "the entirety of 60/068,562 provides adequate written description for a target inhibition method in *C. elegans* with an expression vector that synthesizes two separate complementary RNA strands that form an RNA duplex, wherein the synthesis of the two RNA strands is driven by bacteriophage polymerase promoters such as T3, T7, and SP6 promoters."

18. I respectfully disagree with the statements of the Examiner made in the Office Action as referenced in paragraphs 16 and 17. I have reviewed the Fire provisional application (US 60/068,562) that Fire et al. (WO99/32619) claims priority to. Nowhere in that provisional application does Fire et al describe a target inhibition method in *C. elegans* wherein there is simultaneous synthesis of two RNA strands in a bacterium using a vector that has a DNA sequence inserted between two bacteriophage polymerase promoters, to produce double stranded RNA in the bacterium for the target inhibition method in *C. elegans*.

19 The Fire provisional application describes on page 11 that "[t]he use and construction of an expression vector are known in the art", citing to several references from 1990 and 1991, and a 1997 PCT published application. The 1990 and 1991 references are standard laboratory manuals. The 1997 PCT published application is directed to methods of transforming plant tissue using a bacterium such as *Agrobacterium tumefaciens*. The plasmids described on pages 4-5 of this 1997 PCT application contain different genes (one gene of interest and one antibiotic resistance gene for selection) driven by different promoters. None of the plasmids described in this 1997 PCT application are used to produce RNA corresponding to both strands of a gene sequence in a bacterium.

20. Therefore none of the references cited on page 11 of the Fire provisional application provide any description of a "target inhibition method" wherein a bacterium is used that harbors a plasmid that is producing RNA corresponding to both strands of a gene resulting in the production of double stranded RNA without any human intervention. The "target inhibition method" described in the Fire provisional application required human intervention to carry out *in vitro* transcription, including the following steps: cutting the plasmid with restriction enzymes

(this must be done twice, to produce two plasmids that are capable of making only one RNA strand); purifying the two different cut plasmids; adding the respective bacteriophage polymerases to the two different purified cut plasmids; collecting the resulting single stranded RNA molecules obtained in separate polymerase reactions; and mixing the single stranded RNA molecules to obtain the double stranded RNA, which is then injected in *C elegans* to obtain target inhibition in *C. elegans*.

21. Thus the Examiner's statements that Fire describes a target inhibition method in *C elegans* that uses the claimed invention, that is by using an expression vector that synthesizes in a bacterium two separate complementary RNA strands that form an RNA duplex in the bacterium, wherein the synthesis of the two RNA strands is driven by bacteriophage polymerase promoters such as T3, T7 and SP6 promoters is not supported in the Fire provisional application.

22. It is my opinion that the person of ordinary skill in the art would not have had a reasonable expectation of success, based on the cited combinations of prior art references, of practicing the invention claimed in the present application, of using the type of vectors known in the art to express double stranded RNA "inside" a microorganism, feeding such a microorganism that expresses the double stranded RNA "inside" its cell wall to *C. elegans*, and exerting an effect, from the inner content of the bacterium towards the cell cytoplasm of *C. elegans* cells.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the above-referenced application or any patent issued therefrom.

Date: December 22, 2010

Erwin Sablon, Ph.D

Exhibit 1

Resume (CV) April 2009

Name: Erwin RM Sablon

Age: 44

Birth Date: December 6th 1964

Birth Place: Merchtem, Belgium

Marital Status: Married to Kristien Van Hoof (Highschool teacher, born February 15th 1969)

Children: 1 (Lara Sablon, born March 22nd 1998)

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University studies:

1982-1986: Master in Biology with specialization in Biotechnology: University of Ghent, Belgium

1986-1990: PhD in Molecular Biology, Laboratory of Molecular Biology, University of Ghent, Belgium (Head: Prof. Dr. Ir. Walter Fiers)
"Development of detection technology for the fast analysis of mutants for the determination of structure-function relationships of proteins"

Main technologies used: prokaryotic protein expression, protein engineering, mutagenesis, in vitro transcription, molecular DNA and RNA engineering.

2007-2009: Ongoing. International Master in Business Administration (MBA) following the English-language, part-time executive program at the Vlerick Leuven-Gent School of Management

Professional Career:

1990-1991: Scientific collaborator, Department of Microbiology, R&D, Innogenetics NV

Main technologies used: prokaryotic and eukaryotic protein expression, mutagenesis, in vitro transcription, molecular DNA and RNA engineering.

1992-1995: Scientist: Group Leader Microbial Expression Technology and Protein Engineering, Department of Microbiology, R&D, Innogenetics NV

Main technologies used: prokaryotic and eukaryotic protein expression, mutagenesis, in vitro transcription, molecular DNA and RNA engineering, protein and antibody engineering.

1996-1998: Senior Scientist: Department Head, Department of Microbiology and Bioprocessing, R&D, Innogenetics NV

1999-2001: Senior Scientist: Program Manager Hepatitis Diagnostics, Infectious Diseases Unit, Diagnostics R&D, Innogenetics NV

2002-2004: Senior Scientist: Head Hepatology Group, Infectious Diseases Unit Diagnostics R&D, Innogenetics NV

2005: Senior Scientist: Head Virology Group, Infectious Diseases Unit Diagnostics R&D, Innogenetics NV

2006- May 2008:Principal Scientist: Head Infectious Diseases Unit Diagnostics R&D, Innogenetics NV

June 2008-September 2008: Associate Director Delivery Systems, Ablynx NV

October 2008 – current: Director Project Management, Ablynx NV